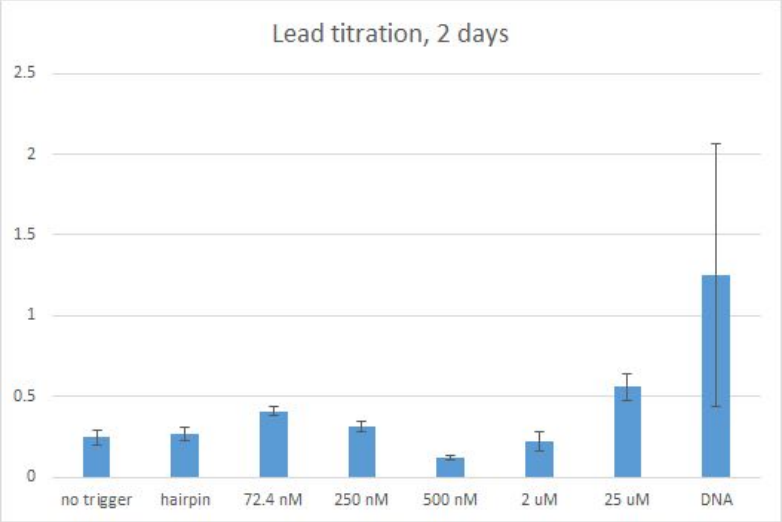
iGEM 2016: Team Pittsburgh
Week 21 Lab Notebook

Monday, October 10

Liquid cultures of lacZ mutagenesis (Maya)
Linearize RBS-T3 with XbaI and EcoRI (Maya)
Anneal PT3 (Maya)
Saturday's cell-free reactions darkened and evaporated-- +10 uL H2O and read (Claire)



Tuesday, October 11

Re-streak cells (Claire)
CMU codon-optimized proteins, CM
G switch, kanamycin
Competent cells, LB

Wednesday, October 12

Liquid cultures (Claire)
Codon-optimized proteins: 4 each (dense growth, difficult to pick individuals)
Competent cells: 4
G switch: 8
50 mg/mL kanamycin sulfate

Thursday, October 13

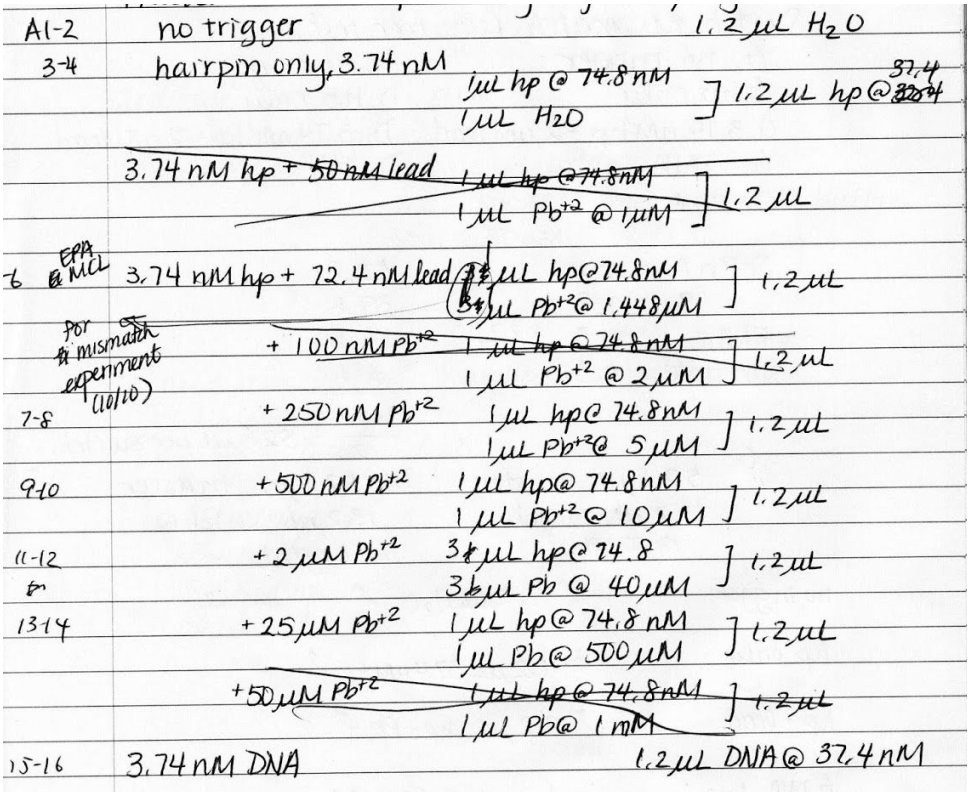
Lacz Mutagenesis with Q5
Linearize PT7-Toehold with EcoRI and PstI
Scan overnight cultures of proteins (Claire with Cheryl)
Time course of proteins, 1 of each overnight culture diluted 1:50
Miniprep G switch (Claire)

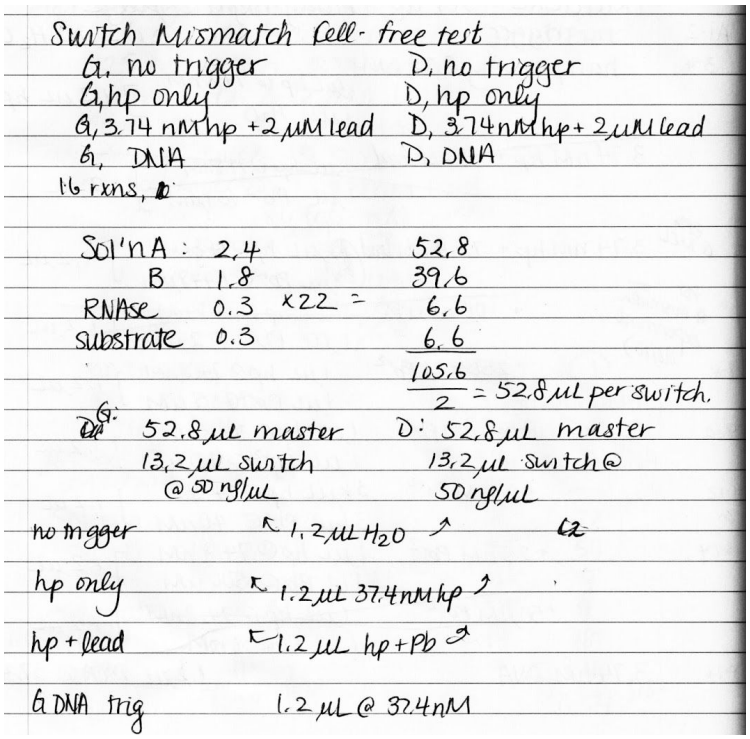
Friday, October 14:

Ligate

PT3 to RBS-T3

Cell-free lead titration and mismatch test





Friday, October 14.
Cell-free lead titration & mismatch (pp110+112)

28 rxns (14 conditions)

Sol'n A	2.4		76.8
B	1.8	x 32	57.6
RNAse	0.3		9.6
substrate	0.3		9.6
			153.6 → D

G

MM = ~~134.4~~ 115.2 (12 cond). master mix ~~19.2~~ 38.4 µL (4 cond. rxns)
59.7 ng/µL switch ~~4.68 µL (25 ng)~~
H_2O ~~4.84~~ µL
50 ng/µL switch
25 µL 59.7 ng/µL + 4.85 µL H_2O = 50 ng/µL

~~50 ng/µL switch = 14.4 µL~~
63.1 ng/µL switch = 9.51 µL
H_2O = 4.89 µL

10.8 µL/rxn

10.8 µL / rxn
- no trigger (1.2 µL H_2O)
- 3.74 nM hp
- 3.74 nM hp + 2 µM lead

Forgot 72.4 nM condition

put tubes in incubator for ~2 min, removed to add DNA to DNA trig. cond, replace in 37. 2hr. 40min.

G no trigger	A	17-18
hairpin	A	19-20
50 nM Pb	A	21-22
100 nM	A	23-24
250 nM	B	1-2
500 nM	B	3-4
2 µM	B	5-6
25 µM	B	7-8
50 µM	B	9-10
DNA	B	(11)-12 low volume
D no	B	13-14
hairpin	B	15-16
2 µM Pb	B	17-18